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Identification of Genes Essential to Long-Chain Alkene Biosynthesis in *Micrococcus luteus*

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Aliphatic hydrocarbons are appealing targets for advanced cellulosic biofuels, as they are predominant components of petroleum-based gasoline and diesel fuels and thus would be compatible with existing engines and fuel distribution systems. We have studied alkene biosynthesis in *Micrococcus luteus*, a close relative of *Sarcina lutea* (now *Kocuria rhizophila*), which was previously reported to biosynthesize *iso*- and *anteiso*-branched, long-chain alkenes. The underlying biochemistry and genetics of alkene biosynthesis were not elucidated in those studies. We show here that heterologous expression of a three-gene cluster from *M. luteus* (Mlut_13230-13250) in a fatty-acid overproducing *E. coli* strain resulted in production of long-chain alkenes, predominantly 27:3 and 29:3 (no. carbon atoms: no. C=C bonds). Heterologous expression of Mlut_13230 (*oleA*) alone produced no long-chain alkenes but unsaturated aliphatic monoketones, predominantly 27:2, and *in vitro* studies with the purified Mlut_13230 protein and tetradecanoyl-CoA produced the same C_{27} monoketone. Gas chromatography-time of flight mass spectrometry confirmed the elemental composition of all detected long-chain alkenes and monoketones (putative intermediates of alkene biosynthesis). Negative controls demonstrated that the *M. luteus* genes were responsible for production of these metabolites. Studies with wild-type *M. luteus* showed that the expression of Mlut_13230-13250 and 29:1 alkene biosynthesis both corresponded with bacterial population over time. We propose a metabolic pathway for alkene biosynthesis starting with acyl-CoA (or -ACP) thioesters and involving decarboxylative Claisen condensation as a key step, which we believe is catalyzed by OleA. Such activity is consistent with our data and with the homology of Mlut_13230 (OleA) to FabH (β-ketoacyl-ACP synthase III), which catalyzes decarboxylative Claisen condensation during fatty acid biosynthesis.

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